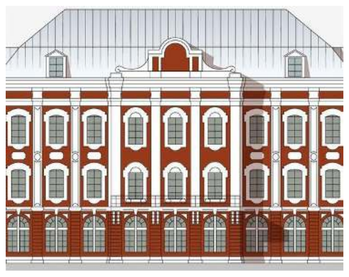
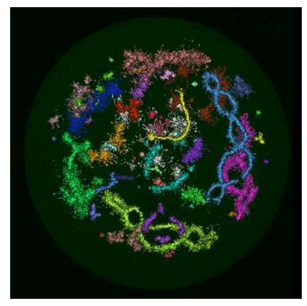


# A complex approach to study the loci of nuclear domains formation



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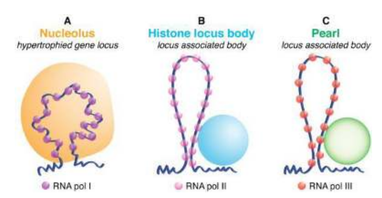
**Introduction:**

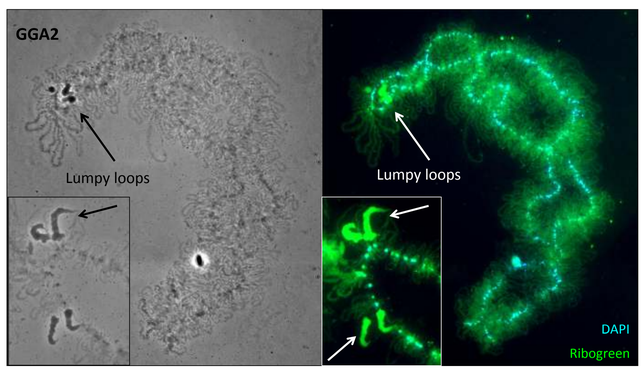
Nuclear domains are categorized into two main groups: those assembled free in the nucleoplasm and those formed at specific chromosome regions as a result of their activity. Here, we used two strategies to analyze the loci of nuclear domains formation on giant lampbrush chromosomes typical for avian and amphibian oocyte nucleus. The first approach is based on successive FISH-mapping the BAC-clones containing genomic fragments with known chromosome position close to the site of nuclear domains formation. The second one is based on mechanical microdissection the chromosome regions at the base of nuclear structure appearance. The DNA from dissected material amplified by DOP-PCR is then subjected to FISH-mapping and high throughput sequencing (NGS). FISH-mapping confirmed the effective generation of the locus-specific probes marking the sites of nuclear structures formation on isolated chromosomes. Sequenced fragments were aligned to the reference genome to precisely identify the loci responsible for the nucleation of the nuclear domain. Additionally, we adopted the approach to isolation and amplification the sequences from RNA-component of nuclear structures associated with lampbrush chromosomes.

## Nuclear bodies and their relationship to gene loci




Nizami, Gall, 2012

## Lampbrush chromosomes (LBCs) – meiotic bivalents from growing oocytes




## Lampbrush chromosomes as initial material for microdissection

Indentification of chromosomal loci of marker structures formation

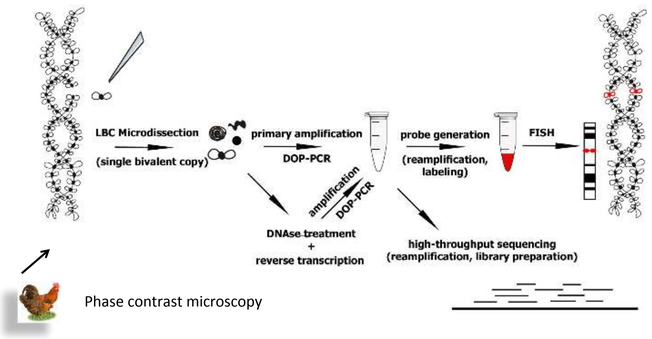
Microdissection

BAC-mapping

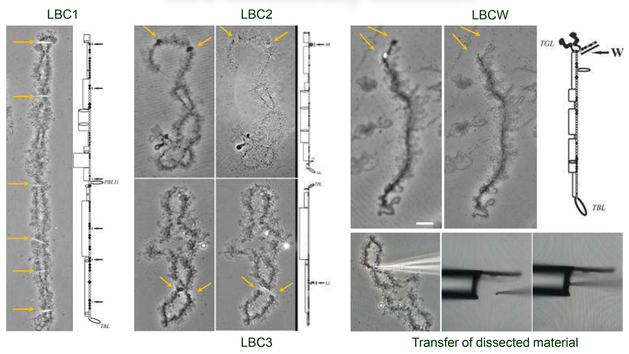
- 18 samples (chromosomal regions) were dissected from lampbrush chromosomes
- for all samples, specific DNA/cDNA probes were generated and verified by FISH
- 6 samples were subjected to high-throughput sequencing procedure followed by bioinformatic analysis.

## The resumptive scheme illustrating the developed workflow

Chicken (*Gallus g. domesticus*) lampbrush chromosome microdissection

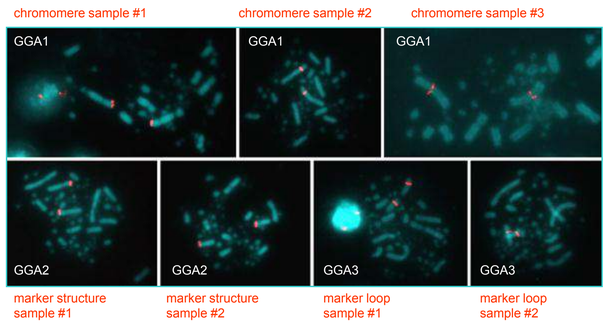



## Different regions of lampbrush chromosomes were successfully dissected



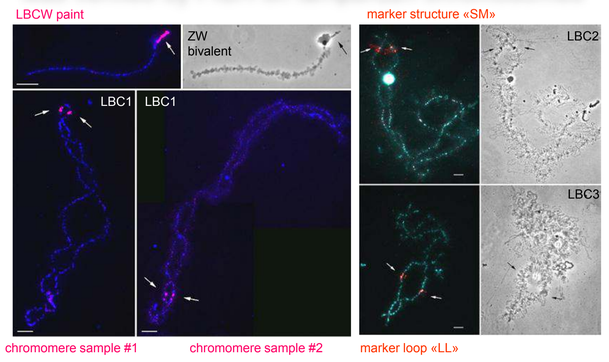

- Starting material: only one bivalent copy
- Targets: regions as small as a single chromomere (~ 2 Mb in size) and individual transcription units (lateral loops)

## The specificity of the DNA-probes generated by microdissection was verified by FISH on metaphase chromosomes



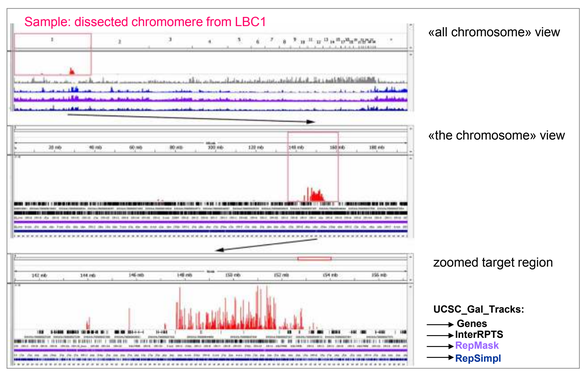

- Bright and specific hybridization signals were detected on corresponding pairs of homologous metaphase chromosomes.

## The specificity of the DNA-probes generated by microdissection was verified by FISH on lampbrush chromosomes

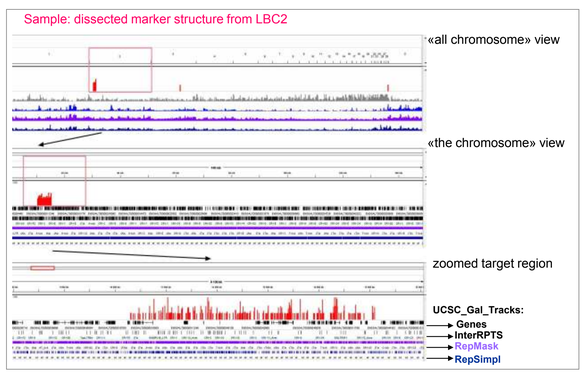



- Bright and specific hybridization signals were detected in corresponding loci of lampbrush chromosomes.

## Assignment of dissected chromosomal regions to chicken genome using high-throughput sequencing




- Visualization of sequencing data via Integrative Genomics Viewer (IGV) browser. Based on mapping results, the precise genomic position, extent and sequence content were determined for the dissected loci.

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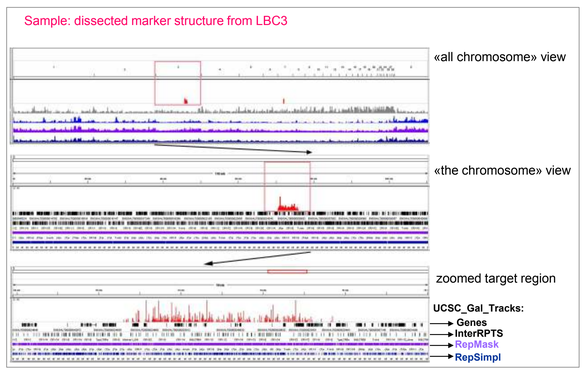
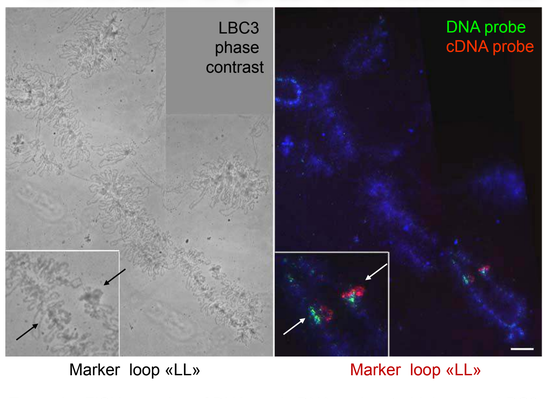


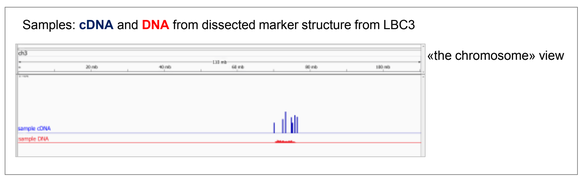

- Visualization of sequencing data via Integrative Genomics Viewer (IGV) browser. Based on mapping results, the precise genomic position, extent and sequence content were determined for the dissected loci.

## Co-hybridization of DNA- and cDNA-probes generated from the same lampbrush chromosome locus




- Two-color FISH-mapping of DNA- and cDNA-probes to LL locus on LBC3

## Assignment of sequenced DNA- and cDNA-probes generated from the same lampbrush chromosome locus to chicken genome




- Visualization of sequencing data via Integrative Genomics Viewer (IGV) browser.

## Conclusions:

- Lampbrush chromosome microdissection offers great prospects for detailed exploration of functionally significant chromosomal regions including individual transcription units.
- Developed comprehensive approach allowes to obtain DNA and RNA samples from particular lampbrush chromosome loci, to define precisely the genomic position, extent and sequence content of dissected regions.
- Using combination of mechanical microdissection, FISH and NGS techniques we defined precisely the loci for marker structures formation on chicken lampbrush chromosomes 2 and 3.
- Our data suggest that large DAPI-positive chromomeres of chicken lampbrush chromosome arms are characterized by low gene density and high repeat content.